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Design of a 3_{10}-helical conformation using α,β-dehydro-residues : Synthesis and solution conformation of Boc-Phe-ΔPhe-Phe-ΔPhe-Phe-OCH_3 (I) and Ac-ΔPhe-Phe-Ala-ΔPhe-Val-OCH_3 (II)

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Abstract : The peptides Boc-Phe-ΔPhe-Phe-ΔPhe-Phe-OCH_3 (I) and Ac-ΔPhe-Phe-Ala-ΔPhe-Val-OCH_3 (II) were synthesized to determine the patterns of conformations in solutions of the sequences containing more than one ΔPhe-residues. The conformational investigations were carried out using 400 MHz and 500 MHz NMR spectroscopy. The deductions were based on the intramolecularly hydrogen bonded NH groups and Nuclear Overhauser Effect (NOE) studies. The temperature and solvent dependence studies were performed in $(CD_3)_2SO$ and $CDCl_3$-$(CD_3)_2SO$ mixture. The results of these investigations indicate that the peptides (I) and (II) adopt similar conformations. The conformations of the peptides are also found to be identical in both $CDCl_3$ and $(CD_3)_2SO$. The observations further indicated the presence of three intramolecular hydrogen bonds of the type $(i + 3) \rightarrow i$ in both peptides with a resulting 3_{10}-helical conformation. These results clearly confirm that the sequences of ΔPhe residues separated by one or more saturated residues adopt a 3_{10}-helical conformation in solution.

Keywords : Dehydro-peptides, 3_{10}-helical conformation, NMR

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1. Introduction

The role of α,β-dehydro-residues in the design of specific peptide structures is fast becoming a useful tool as definite trends are emerging for the dehydro-residue based conformations. A dehydro-residue introduces strong constraints which allow the formation of only a few specific conformations in peptides [1–43]. The magnitude of constraints caused by a dehydro-residue is dependent on the size and geometry of its side chain.

According to the magnitude of constraints, the dehydro-residues can be divided into three broad categories :

(i) the residues whose side chains do not branch at C^{β} such as Phe, Leu, Trp, Tyr *etc* adopt only three possible conformations which correspond to three sets of (ϕ, ψ) torsion angles centred at –60, 140; 80, 0 and –60, –30° and their enantiomeric structures [3–38].

(ii) the residues, in which the branching occurs at C^{β} atom such as Val and Ile, produce the strongest steric effects with (ϕ, ψ) torsion angles centred at ± 60, ± 30° [39].

(iii) the ΔAla with-CH_2 as the side chain induces mild steric effects and adopts an extended conformation with ϕ, ψ torsion angles centred at ± 180, ± 180° [40,42].

The peptides containing dehydro-residues of category (i) have been extensively studied [1–38]. The constraints introduced by these dehydro-residues favour the formation of a β-turn II conformation when substituted at (i + 2) position of a tetrapeptide [15–28]. The incorporation of these residues in peptides at (i + 1) position also produces a β-turn II conformation if the (i + 2) position of the sequence has a flexible Gly or Ala residue [1–3, 7] whereas, a bulky residue at (i + 2) position forces the peptide to adopt an alternating right-handed and left-handed *S*-shaped conformation with (ϕ, ψ) torsion angles centred at ± 50, ± 40° [11–14]. If there are more than one dehydro-residues separated by one or more saturated residues, the resulting conformation is a 3_{10}-helix [29–36]. These rules are based on the investigations carried out in the crystalline state [3–38]. Such a generalisation has not yet been obtained in the solution state where only limited data are available [43–47]. Therefore, we have synthesized the Boc-Phe-ΔPhe-Phe-ΔPhe-Phe-OCH_3 (I) and Ac-ΔPhe-Phe-Ala-ΔPhe-Val-OCH_3 (II) and analysed their conformations in solution using 2-dimensional NMR.

2. Experimental procedures

2.1. Peptide synthesis :

The peptides were synthesized in solution phase using azlactone method [48,49]. The reactions were monitored by thin layer chromatography using (a) and (b) solvent systems . (a) Chloroform ($CHCl_3$) : Methanol (MeOH) = 9 : 1; (b) *n*-Butanol (*n*-BuOh) : Acetic acid (AcOH) : H_2O = 4.5 : 0.5 : 0.5.

2.1.1. Peptide 1 :

2.0 g Boc-Phe-OH was reacted with 1.6 g of β-phenyl serine using 0.84 ml of *N*-methylmorpholine (NMM) and 0.98 ml of isobutylchloroformate (IBCF). 11.31 ml of NaOH was used to neutralise β-Phenyl serine. The reaction mixture was worked up and 2.2 g of Boc-Phe-β-Phenyl serine was obtained which gave Rf^a = 0.69, Yield = 76%. Boc-Phe-β-Phenyl serine (3.12 g) was converted to Boc-Phe-ΔPhe-azlactone using 0.62 g of sodium acetate and acetic anhydride with Rf^a = 0.90; Yield = 75%. Boc-Phe-ΔPhe-azlactone was hydrolysed using 1 equivalent NaOH at pH 8 to Boc-Phe-ΔPhe-OH. 1.4 g of

Boc-Phe-ΔPhe-OH was coupled with 1.1 g of L-Phe-OMe.HCl (neutrallised with 0.56 ml NMM) by mixed anhydride method using 0.37 ml of NMM and 0.45 ml of IBCF. After work up and isolation, the powder like compound Boc-Phe-ΔPhe-Phe-OCH_3 (A) was obtained on evaporation of the ethylacetate layer. Rf^a = 0.69, Rf^b = 0.90; Yield = 60%; M.P. = 132°C.

2.0 g of Boc-Phe-ΔPhe-Phe-OCH_3 (A) was deprotected using 1.4 ml TFA/DCM mixture (1 : 1) for 45 minutes. Excess DCM and TFA were removed under vacuum. The residual mixture was triturated with diethylether and kept in the refrigerator and a white precipitate was decanted and dried to yield-2.24 g of TFA-Phe-ΔPhe-Phe-OCH_3 (B).

1.2 g of Boc-Phe-ΔPhe-OH was dissolved in tetrahydrofuran and 0.32 ml of NMM and 0.38 ml of IBCF were added to the solution and the reaction mixture, stirred at –10°C in an ice salt bath for 20 minutes. 1.7 g of TFA-Phe-ΔPhe-Phe-OCH_3 was dissolved in THF and neutralised with 0.4 ml NMM and added to the reaction mixture with stirring at 0°C for

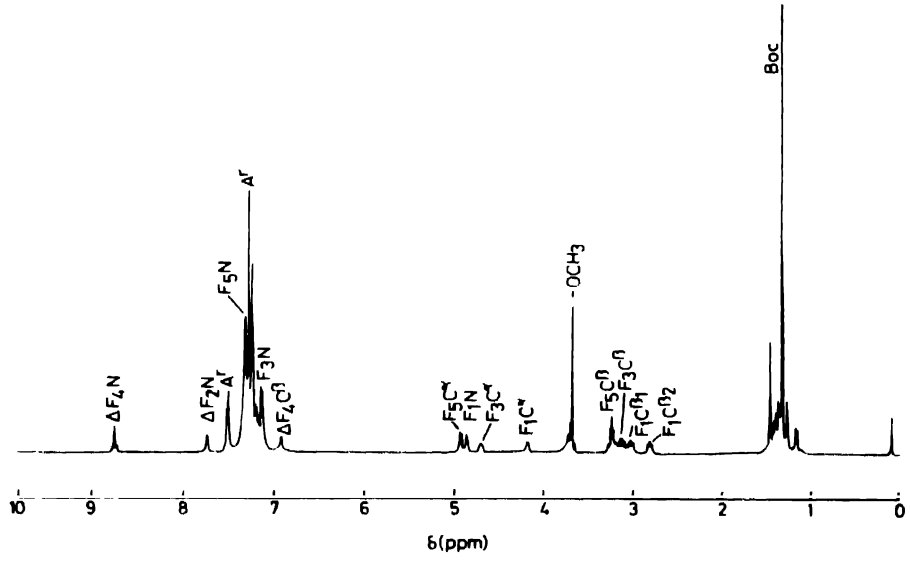

Figure 1(a). 500 MHz ^{1}HNMR spectrum of Peptide(I) in $CDCl_3$

two hours and at room temperature overnight. The THF was removed under vacuum and residual mixture was washed with water, 10% sodium bicarbonate, 5% citric acid. The ethylacetate layer was dried over anhydrous sodium sulphate for two hours. The ethylacetate layer was evaporated to give 1.0 g of required peptide. Rf^a = 0.65, Rf^b = 0.75, Yield = 50%. The NMR spectral assignments for this peptide as observed in $CDCl_3$ (Figure 1a) and in $(CD_3)_2SO$ (Figure 1b) are given in Table 1.

2.1.2. Peptide II :

N-Ac-ΔPhe-OH or α-acetoamido cinnamic acid was coupled with L-Phe-OMe by mixed anhydride method. 2.0 g of N-Ac-ΔPhe-OH (9.0 mmol) was dissolved in THF and coupled

with L-Phe-OMe (obtained by neutralising HCl-Phe-OMe with 1.25 ml of NMM) using 1.08 ml NMM and 1.26 ml of IBCF as described earlier. THF was removed from the reaction mixture under vacuo. The residual solution was dissolved in ethylacetate and washed with water, 10% sodium bicarbonate and 5% citric acid. The ethylacetate layer was

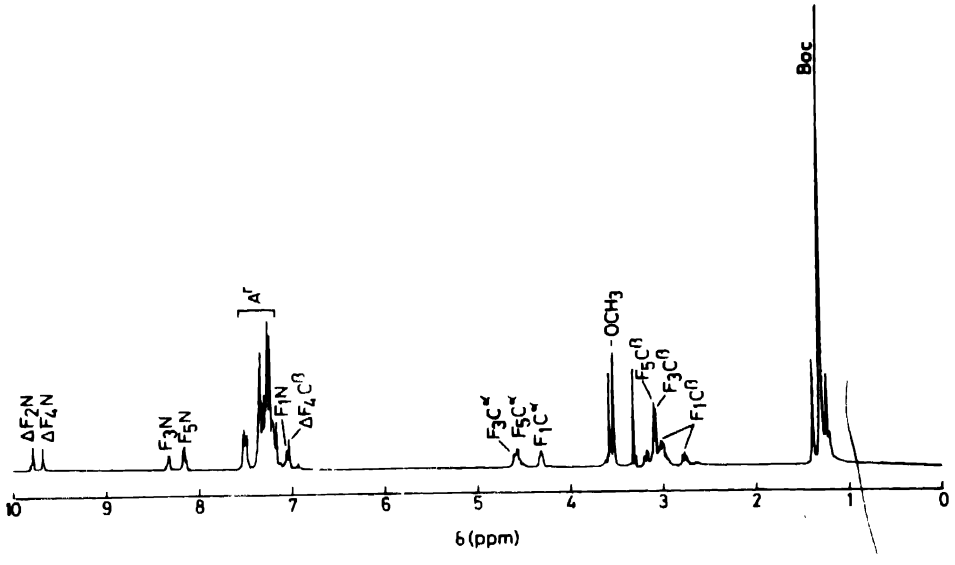

Figure 1(b). 500 MHz ^{1}HNMR spectrum of Peptide(I) in $(CD_3)_2SO$.

dried over sodium sulphate overnight. 1.8 g of N-Ac-ΔPhe-Phe-OCH_3 was acquired on evaporation of ethylacetate layer. This peptide was hydrolysed using 1 equivalent NaOH

Table 1. Spectral assignments of peptides I and II

(a) Peptide residue No.		Boc-Phe-Δ-Phe-Phe-Δ-Phe-Phe-OCH_3 (I) 1 2 3 4 5 Chemical shifts δ (ppm)		
		NH	$C^{\alpha}H$	$C^{\beta}H$
	$CDCl_3$	4.85	4 17	2.81–3.01
Phe1	DMSO	7.07	4 30	2 95
	$CDCl_3$	7.72	–	7.15
ΔPhe2	DMSO	9.79	–	7 02
	$CDCl_3$	7 13	4.69	3.11
Phe3	DMSO	8.32	4.76	3.16
	$CDCl_3$	8.75	–	6.97
ΔPhe4	DMSO	9 69	–	*
	$CDCl_3$	7 30	4.92	3.22
Phe5	DMSO	8.17	4.55	3.08

Table 1. *(Cont'd.)*

(a) Peptide residue No		Boc-Phe-Δ-Phe-Phe-Δ-Phe-Phe-OCH_3 (I) 1 2 3 4 5 Chemical shifts δ (ppm) NH	$C^{\alpha}H$	$C^{\beta}H$
(b) Peptide residue No.		N-Ac-Δ-Phe-Phe-Ala-Δ-Phe-Val-OCH_3 (II) 1 2 3 4 5		
	$CDCl_3$	8.97	–	6.17
ΔPhe1	DMSO	9.82	–	6.60
	$CDCl_3$	8.16	4.30	3.31–3.17
Phe2	DMSO	8.36	4.53	3.02–3.13
	$CDCl_3$	7.75	4.58	1.12
Ala3	DMSO	8.08	4.54	1.26
	$CDCl_3$	8.68	–	6.75
ΔPhe4	DMSO	9.57	–	7.78
	$CDCl_3$	7.40	4.54	2.25
Val5	DMSO	7.78	4.50	2.19

* the resonance was not resolved.

(pH 8.0). 1.2 g of N-Ac-ΔPhe-Phe-OH were obtained. Yield = 53%, Rf^b = 0.86. 2.0 g Boc-Ala-OH was coupled with 2.3 g DL-Threo-β–Phenylserine (neutrallised with 1 eq NaOH) using 1.2 ml NMM and 1.4 ml IBCF. After washing 3.0 g of Boc-Ala-β-Phenylserine was isolated. Rf^a = 0.52; Yield = 96%.

2.0 g of β-Phenylserine was dissolved in acetic anhydride and treated with 0.86 g sodium acetate to yield 2.0 g of Boc-Ala-ΔPhe-azlactone. 2.0 g Boc-Ala-ΔPhe-azlactone was hydrolysed using 5 ml of NaOH to give 3.8 g of Boc-Ala-ΔPhe-OH after washing the reaction mixture and upon evaporation of the ethylacetate layer. 1.86 g of Boc-Ala-ΔPhe-OH was coupled with 2.0 g of L-Val-OMe. HCl (neutralised with 0.7 ml NMM) using 0.6 ml NMM and 0.7 ml IBCF by mixed anhydride procedure as described above. 2.0 g of the desired peptide was obtained after working up the reaction mixture as described in previous steps. Rf^b = 0.9; Yield = 89%.

1.5 g of Boc-Ala-ΔPhe-Val-OCH_3 was deprotected on treatment with 3 ml of (1 : 1) DCM/TFA mixture for 40 minutes at room temperature. The excess TFA and DCM were removed on the rotatory evaporator. The oily residue was triturated with anhydrous diethylether and kept in the refrigerator. White solid product was decanted and dried. The yield of the TFA salt was 1.4 g.

0.6 g of N-Ac-ΔPhe-Phe-OH was dissolved in THF and 0.19 ml of NMM and 0.24 ml of IBCF were added at –10°C with stirring for 20 minutes. 0.98 g of TFA-Ala-ΔPhe-Val-OCH_3 neutralised with 0.24 ml NMM was then added to the reaction mixture and

stirred at 0°C for 2 hours and at room temperature overnight. THF was removed from the reaction mixture and the residual solution was dissolved in ethylacetate and the ethylacetate

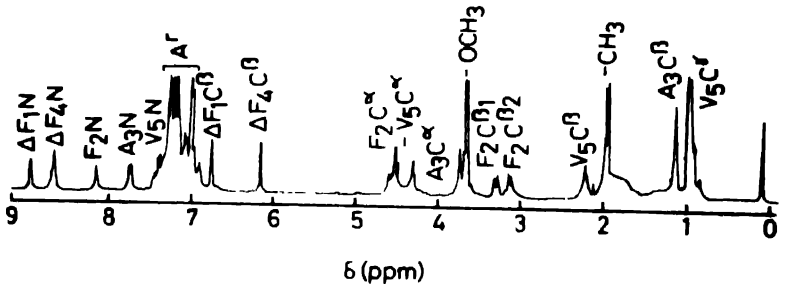

Figure 1(c). 500 MHz ^{1}HNMR spectrum of Peptide(II) in $CDCl_3$

layer was washed with water, 10% NaHCO and 5% citric acid. 0.5 g of N-Ac-ΔPhe-Phe-Ala-ΔPhe-Val-OCH_3 was obtained on evaporation of the ethylacetate layer. Rf^a = 0.57,

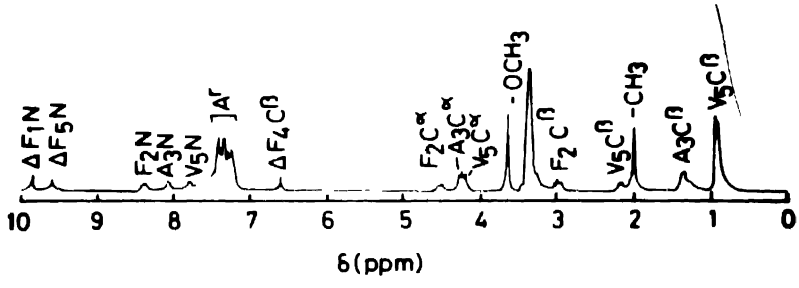

Figure 1(d). 500 MHz ^{1}HNMR spectrum of Peptide(II) in $(CD_3)_2SO$ •

Rf^b = 0.93, Yield = 41%. The NMR spectral assignments as observed in $CDCl_3$ (Figure 1c) and $(CD_3)_2SO$ (Figure 1d) are given in Table 1.

2.2. NMR spectroscopy :

The samples were prepared from lyophilised peptides by dissolving in $CDCl_3$ or $(CD_3)_2SO$. The peptide concentrations used were 10 mM. The ^{1}H NMR spectra for these peptides were obtained on Bruker AMX-400 MHz and Bruker AM-500 MHz machines. The phase sensitive 2D NMR experiments were employed for assignments and for secondary structure elucidation. The 2D NMR spectra were recorded for 512 t_1 values with 1024 complex points for each free induction decay. 32 scans per t_1 point were taken. Both +ve and –ve cross peaks were plotted without discrimination in all DQF COSY [50], NOESY [51] and ROESY [52] spectra. For NOESY experiments the mixing time of 400 ms was used and the ROESY experiments were carried out using a mixing time of 300 ms. The data were processed by zero filling ω_1 to 1K. The raw data were multiplied with suitable window functions *i.e.* phase sensitive sine bell in both dimensions.

3. Results and discussion

3.1. Conformational analysis :

3.1.1. Spectral assignments

Due to the presence of all Phe residues in (I), the sequential NOEs (Figures 2a, 2b, 2c, 2d) were helpful to distinguish the peaks of various pairs of protons. Both ΔPhe2 and ΔPhe4 NHs were easily distinguishable as singlets in the low field region as the C^{α} proton in a dehydro-residue is absent. The ΔPhe C^{β} protons were not easily distinguishable as they are close to the amide proton region. The Phe1 NH appears at a relatively high field position (4.25 ppm) because it is being protected by the Boc group and the urethane linkage [53] (It shifts to 7.08 ppm is $(CD_3)_2$ SO). The ΔPhe2 NH was assigned by virtue of the sequential

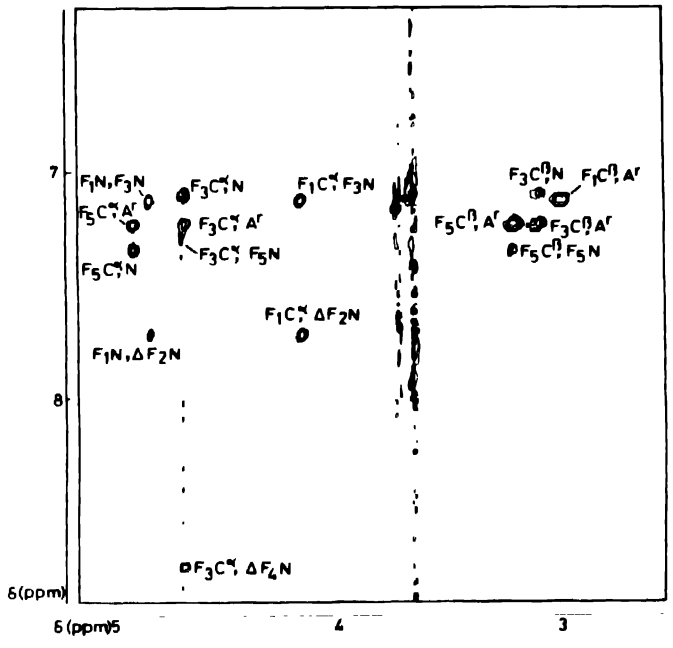

Figure 2(a). NH–C^{α}H region of 500 MHz 2D-ROESY spectrum of Peptide(I) in $CDCl_3$

NOE with Phe1 C^{α}H thus the other ΔPhe NH singlet was assigned as ΔPhe4 NH. The C^{α} proton showing an NOE to it is Phe3 C^{α}H and the corresponding amide is Phe3 NH and the only unassigned NH is Phe5 NH (Figures 2a, 2b, 2c, 2d). Similarly, the assignments were made in $(CD_3)_2$SO. The similar procedure was used to assign for peptide II using sequential NOEs (Figures 3a, 3b, 3c, 3d) in both $CDCl_3$ and $(CD_3)_2$SO.

3.1.2. Analysis of the NOE data :

Rotating frame NOESY (ROESY) experiments were performed as the intensity of the cross peaks in a NOESY spectra are very weak due to the $\omega\tau_c \equiv 1$ condition for small peptides. The NOEs for peptides are a population weighted average of the NOEs in all the

conformational states available to the peptide. For short peptides in solution the d_{NN} (i, i + 1) distance becomes short enough to give observable NOEs between residues with ϕ and ψ angles in the helix region of (ϕ, ψ) space. The d_{NN} distance does not vary much in the

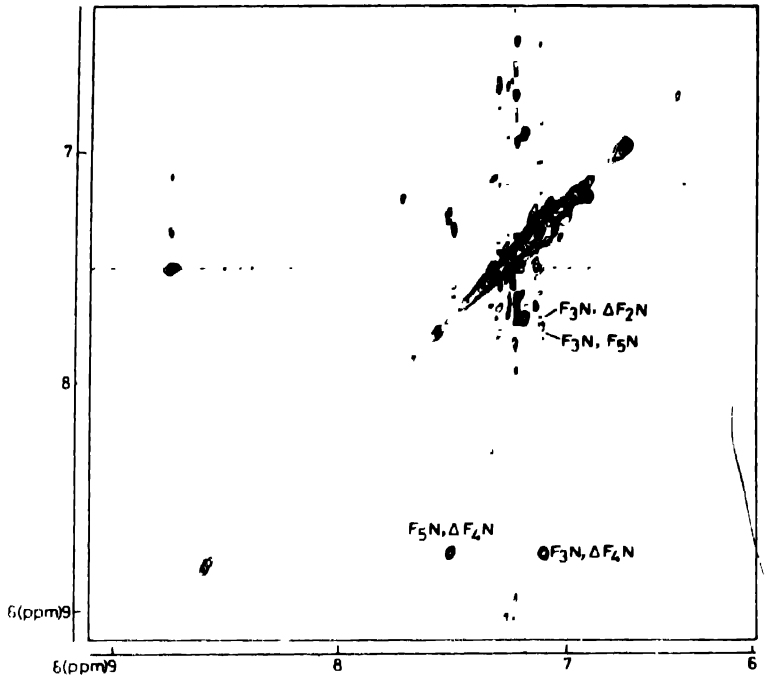

Figure 2(b). NH–NH region of 500 MHz 2D-ROESY spectrum of Peptide(I) in $CDCl_3$.

allowed range of ϕ, ψ torsion angles in this region. Thus the differences in the $N_iH...N_{i+1}H$ NOEs reflect the difference in the population of conformer of helical ϕ, ψ values. The $C_i^{\alpha}H$ ..NH_{i+1} type connectivities used for sequential assignments are indicative of unfolded conformations of the peptides. The observation of equally strong $N_iH...N_{i+1}H$ NOEs is immediately indicative of folded conformations. The observation of medium range NOEs $d_{\alpha N}$ (i, i + 2), $d_{\alpha N}$ (i, i + 3), $d_{\alpha N}$ (i, i + 4), $d_{\alpha\beta}$ (i, i + 4) are strong indications of helical conformations [54]. But in very small peptides such as the ones discussed here, these NOEs are rare because of their small sequences. In such cases the NOE data needs support from the amide accessibilty experiments in order to probe the hydrogen bonding scheme of these peptides.

A. Boc-Phe-ΔPhe-Phe-ΔPhe-Phe-OCH_3 (I)

(i) In $CDCl_3$:

The presence of $N_iH...N_{i+1}H$ NOEs (Table 2) between amides of all the residues indicated that the peptide was predominantly helical in nature [55]. In addition to these NOEs the medium range NOEs of the type $C_i^{\alpha}H...N_{i+2}H$ were observed between Phe1C^{α}H...Phe-

3NH and Phe3C$^{\alpha}$H...Phe5NH (Figure 2a). These distances are possible only for a 3_{10}-helical conformation (d = 3.8 Å), but not for α-helix (d = 4.2 Å) [54,55]. The presence of Phe1NH...Phe3NH and Phe3NH...Phe5NH NOEs is a further confirmation of a 3_{10}-helical structure for this peptide in $CDCl_3$ (Figure 2b).

(ii) In $(CD_3)_2SO$:

The intensity pattern of the NOEs in $(CD_3)_2SO$ (Table 2) is largely similar to the one observed in $CDCl_3$. The $N_i H...N_{i+1}H$ NOEs in $(CD_3)_2SO$ are observed between all the

Table 2. Some important NOEs observed in peptides I and II and comparison with corresponding distances in the crystal structure of a 3_{10}-helical peptide Boc-Phe-Δ-Phe-Val-Phe-ΔPhe-Val-OCH_3 [33].

Peptide	Proton	Connectivity	NOE[a]		distance(Å)[b]
			$CDCl_3$	DMSO	
I	Phe1 NH	ΔPhe2 NH	M	M	2.2–3.0
I	Phe1 C$^{\alpha}$H	ΔPhe2 NH	M	M	2.6–3.4
		Phe3 NH	W	–	3.0–3.5
I	ΔPhe2 NH	Phe2 NH	M	W	2.2–3.0
I	Phe3 NH	ΔPhe4 NH	M	M	2.2–3.0
		Phe5 NH	W	–	
I	Phe3 C$^{\alpha}$H	ΔPhe4 NH	M	M	2.6–3.4
		Phe5 NH	W	–	3.0–3.5
I	ΔPhe4 NH	Phe5 NH	M	M	2.2–3.0
II	ΔPhe1 NH	Phe2 NH	M	M	2.2–3.0
II	Phe2 C$^{\alpha}$H	Ala3 NH	M	M	2.6–3.4
		ΔPhe4 NH	W	–	3.0–3.5
II	Ala3 NH	ΔPhe4 NH	M	–	2.2–3.0
		Phe2 NH	M	M	2.2–3.0
II	Ala3 C$^{\alpha}$H	ΔPhe4 NH	M	M	2.6–3.4
		Val5 NH	W	W	3.0–3.5

[a]NOEs indicated and characterized on the basis of intensity as S–strong, M–Medium, W–Weak. The assessment is done on comparison with intraresidue NOEs in the peptide

[b]Shows the upper and lower limits of the corresponding distances in the crystal structure of the mentioned peptide.

residues in $CDCl_3$ (Figure 2d) in addition to the $C^{\alpha}_iH...N_{i+1}H$ (Figure 2c) which are expected for a helical conformation but the medium range NOEs are not observed in $(CD_3)_2SO$. This could be due to the effect of viscosity of a more viscous solvent $(CD_3)_2SO$ on the $\omega\tau_c$ correlation which could result in a decrease in the upper limt of the observable distance in the spectra [56].

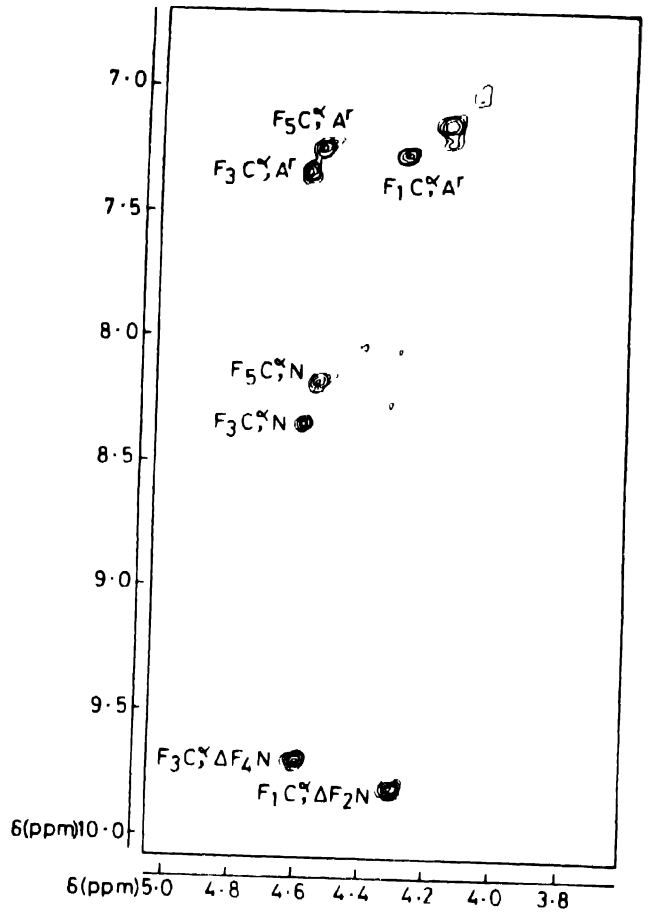

Figure 2(c). NH-$C^{\alpha}H$ region of 500 MHz 2D-ROESY spectrum of Peptide(I) in $(CD_3)_2SO$

B. Ac-ΔPhe-Phe-Ala-ΔPhe-Val-OCH_3 (II)

(i) In $CDCl_3$:

As seen from Figure 3b the strong $N_iH...N_{i+1}H$ NOEs are observed throughout the peptide chain indicating a 3_{10}-helical conformation [54]. All the sequential NOEs were observed ΔPhe1 NH. Phe2 NH, Phe2 NH. .Ala3 NH, Ala3 NH...ΔPhe4 NH and ΔPhe4 NH...Val5 NH (Figure 3b). In addition to these NOEs the medium range NOEs of the type $C_i^{\alpha}H...N_{i+2}H$ were also observed between Phe2 $C^{\alpha}H$...ΔPhe4 NH and Ala3 $C^{\alpha}H$...Val5 NH (Figure 3a). Since the ΔPhe C^{α} proton is absent thus more of these NOEs are not observed. These NOEs are indicative of the existence of a 3_{10}-helix ($d \equiv 3.8$ Å) and not of an α-helix ($d \equiv 4.2$ Å) [54,55]. The $N_iH...N_{i+2}H$ distance is around 4.2 Å for both these structures but since this is not observed it can be inferred that the upper limit of the NOE distances

observed here is around 4.0 Å. The NOE pattern observed for this peptide indicates the existence of a 3_{10}-helical conformation.

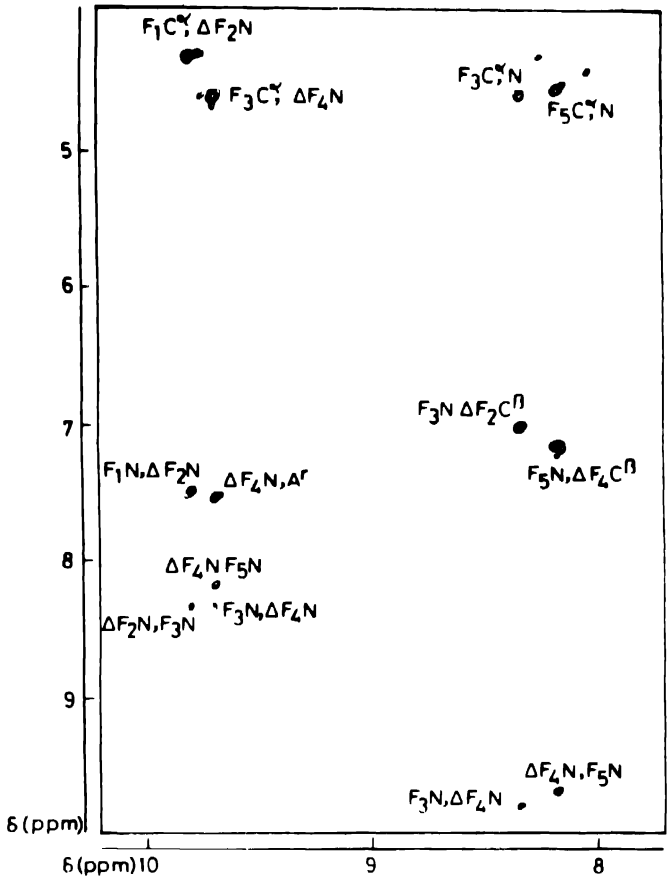

Figure 2(d). NH–NH region of 500 MHz 2D-ROESY spectrum of Peptide(I) in $(CD_3)_2SO$.

(ii) In $(CD_3)_2$ SO :

Peptide (II) in $(CD_3)_2SO$ shows a similar NOE pattern (Table 2) as observed in peptide (I). The following NOEs of the type $N_iH...N_{i+1}H$ are observed ΔPhe1NH...Phe2NH, Phe2NH ..Ala3NH, Ala3NH...ΔPhe4NH and Phe4NH...Val5NH (Figure 3d) in addition to the $C^{\alpha}_iH...N_{i+1}H$ NOEs (Figure 3c). The strong $C_i^{\alpha}H...N_{i+1}H$ and $N_iH...N_{i+1}H$ type NOEs are observed for all residues. The presence of the $N_iH...N_{i+1}H$ NOEs is a strong indication of helical conformation. Thus, the conformation of peptide II is quiet similar to that of peptide (I). This suggests that the peptide (II) also adopts a 3_{10}-helical conformation in $(CD_3)_2SO$.

3.1.3. Amide accessibility :

In order to determine the hydrogen bonding in peptides, the accessibility of amide protons to the solvent was investigated by temperature studies and solvent titration experiments in $(CD_3)_2SO$ and $CDCl_3$-$(CD_3)_2SO$ mixture respectively.

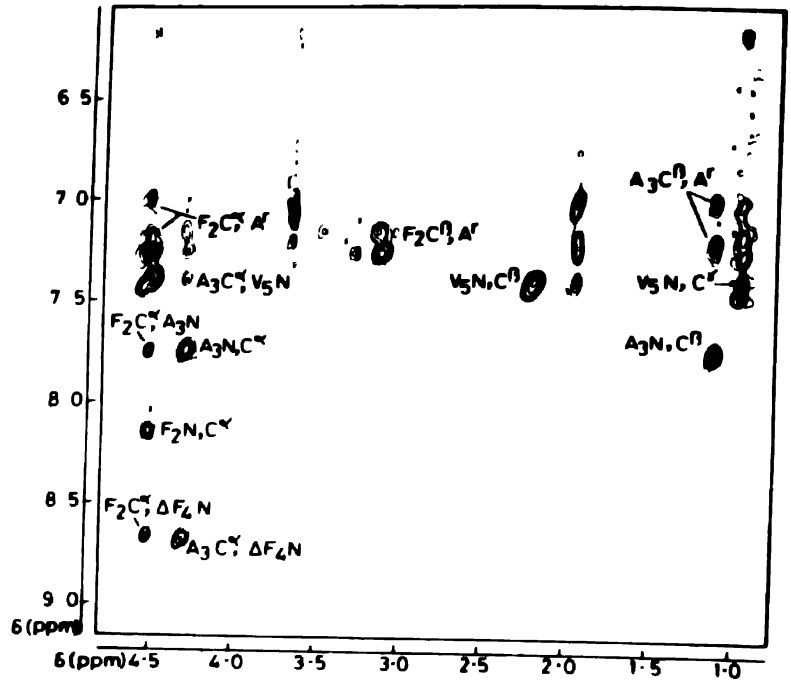

Figure 3(a). NH–$C^{\alpha}H$ region of 500 MHz 2D-ROESY spectrum of Peptide(II) in $CDCl_3$.

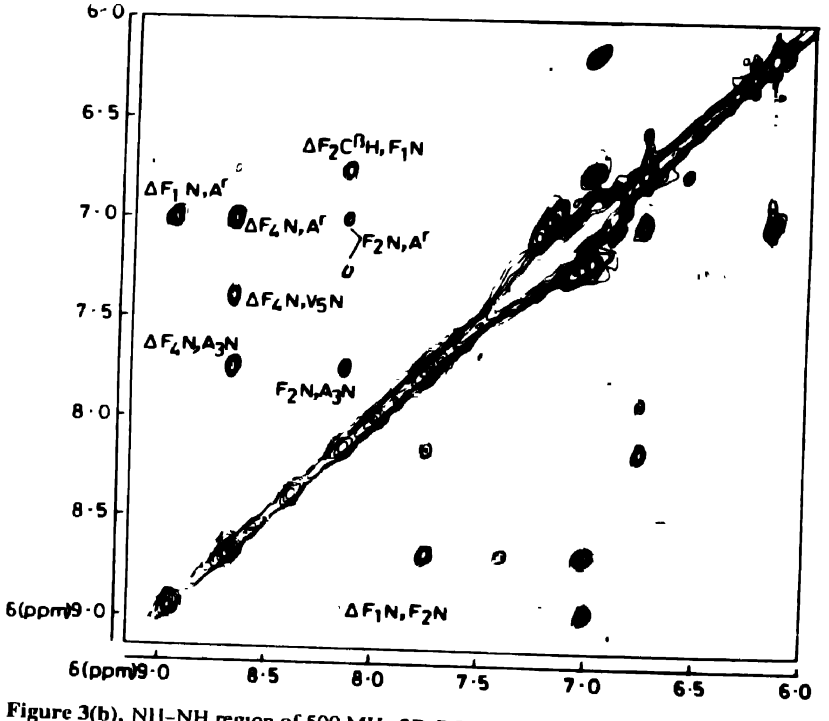

Figure 3(b). NH–NH region of 500 MHz 2D-ROESY spectrum of Peptide(II) in $CDCl_3$

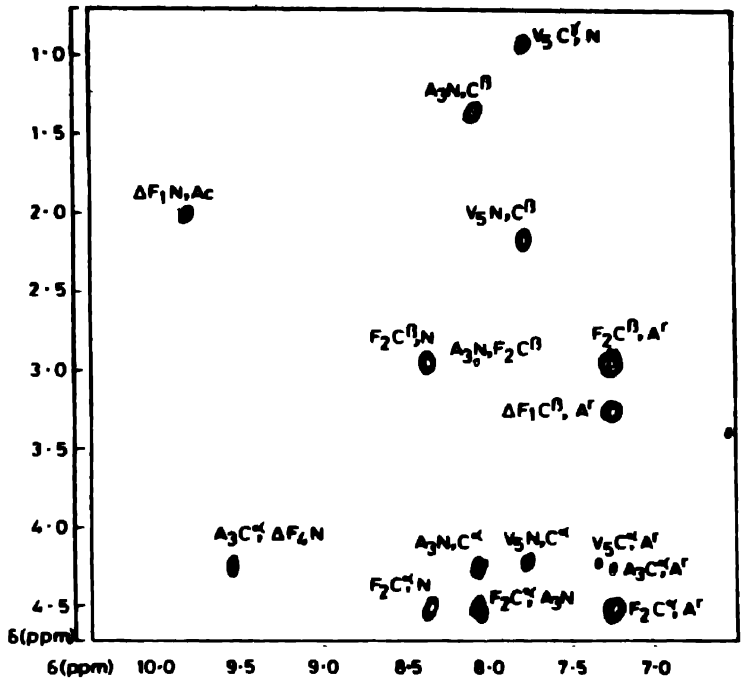

Figure 3(c). NH–C^{α}H region of 400 MHz 2D-ROESY spectrum of Peptide(II) in $(CD_3)_2SO$.

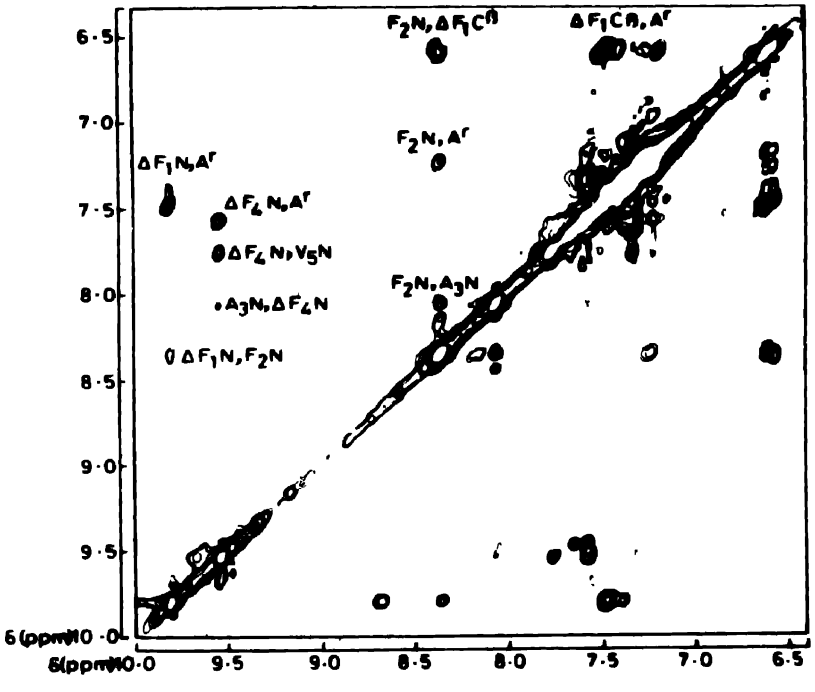

Figure 3(d). NH–NH region of 400 MHz 2D-ROESY spectrum of Peptide(II) in $(CD_3)_2SO$.

3.1.4. Temperature dependence studies :

The results of the temperature studies (Table 3) in a solvating media such as $(CD_3)_2SO$ suggested that the Phe1 and ΔPhe2 in peptide (I) and ΔPhe1 and Phe2 in (II) are solvent

Table 3. Amide resonances, temperature coeffecients and coupling constants.

NH resonance (residue)		δ (ppm)		$d\delta/dT$ (ppm/°K)	J (Hz)	
		$CDCl_3$	DMSO	DMSO	$CDCl_3$	DMSO
Phe1	I	4 25	7.08	0.009	4.70	7 75
ΔPhe2	II	8 98	9 82	0.006	–	–
ΔPhe2	I	7 72	9 80	0 007	–	–
Phe2	II	8.15	8 36	0.007	–	8 40
Phe3	I	7 13	8 33	0.0045	6 90	6 15
Ala3	II	7.75	8.08	0 0035	6 70	4 50
ΔPhe4	I	8.75	9 69	0 0040	–	–
ΔPhe4	II	8 67	9.56	0 0038	–	–
Val5	I	7 30	8.17	0.004	3 45	7.20
Phe5	II	7 40	7 80	0 0037	–	7 70

exposed as they show high $d\delta/dT$ values ($\geq 7 \times 10^{-3}$ ppm/°K) whereas the remaining three residues in both peptides seem to be solvent sheilded because the temperature coefficients are in the range $4–5 \times 10^{-3}$ ppm/°K [57]. Thus Phe3 NH, ΔPhe4 NH, Phe5 NH in peptide (I) and Ala3 NH, ΔPhe4 NH, Val5 NH in peptide (II) seem to be involved in intramolecular hydrogen bonding. It is also indicative of the formation of a 4 → 1 hydrogen bond as observed in the 3_{10}-helical conformation. Some of the $d\delta/dT$ values are intermediate, suggesting the existence of weaker hydrogen bonds [58]. This phenomenon has also been observed in the case of crystal structures of small helical peptides [34]

3.1.5. Solvent titration studies :

The change in the chemical shifts was monitored on increase in the concentration of a polar solvent $(CD_3)_2SO$ in the solution of the peptides in $CDCl_3$. As evident from the curves in Figures 4a and 4b, the changes for Phe3 NH, ΔPhe4 NH, Phe5 NH in peptide (I) and Ala3 NH, ΔPhe4 NH, Val5 NH in peptide (II) are nominal as compared to the other amides in these peptides This suggests that these amide protons are solvent shielded and are involved in a 4 → 1 type of hydrogen bonding [59]. Thus both the temperature dependence and solvent titration studies indicate hydrogen bonding scheme compatible with 3_{10}-helical structure (Figure 5).

3.1.6. Spin-spin coupling constants :

The spin-spin coupling constants are crude indicators of the conformation of small peptides but some idea of ϕ values can be obtained from the ^{3J}NH C$^{\alpha}$H a values for different amides. The vicinal coupling constants have an expected value of 4.2 Hz for 3_{10}-helix ($\phi = -60°$) [60]. The coupling constants for Phe1 NH and Phe5 NH are very small (≈ 4 Hz) which suggest

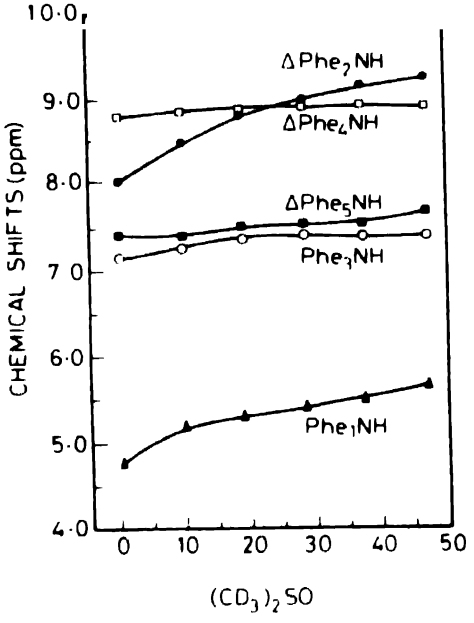

Figure 4(a). Solvent titration curve of Peptide(I)

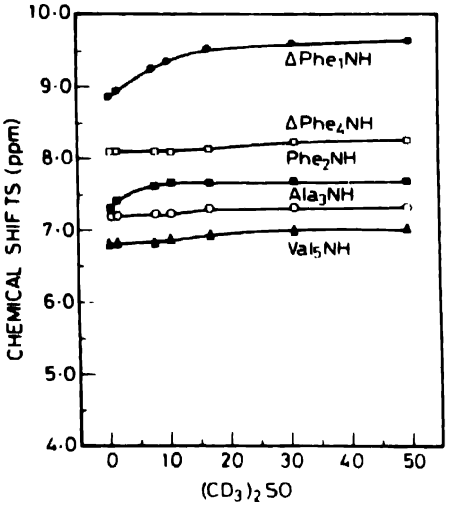

Figure 4(b). Solvent titration curve of Peptide(II)

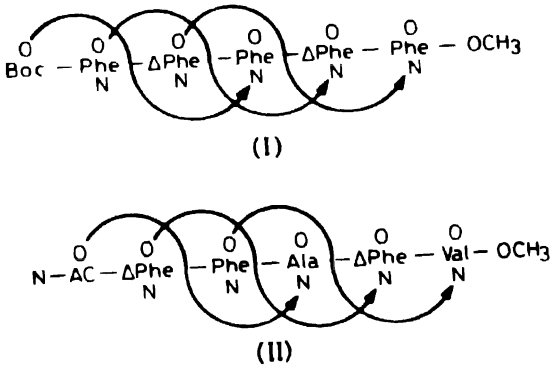

Figure 5. The hydrogen bonding scheme in Peptides(I) and (II).

a ϕ value of −65° indicating the peptide to be folded. In $(CD_3)_2SO$, these values are intermediate for both peptides I and II (≈ 7.0 Hz) which can be considered in the range of helical conformation ($\phi = -70°$).

4. Conclusions

The results of these investigations suggest that

1. The conformations of ΔPhe containing peptides are similar in both polar and nonpolar media.
2. The sequences containing more than one ΔPhe residues which are seperated by a saturated residue adopt a 3_{10}-helical conformation in solution.
3. The sequences containing more than one ΔPhe residues which are seperated by two saturated residues, also result in a 3_{10}-helical structure in solution.
4. The ΔPhe containing peptides adopt similar structures in solution as well as in the solid state [29-36].

These results indicate that the ΔPhe is a strong inducer of specific secondary structures in peptides. Therefore the ΔPhe residue offers a powerful design tool to produce specific conformations in peptides.

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